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Review Article

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EPR effect based nanocarriers targeting for treatment of cancer

Kavita R Gajbhiye[1]*, JM Gajbhiye[1]*

*Corresponding author:

Kavita R Gajbhiye
JM Gajbhiye

[1]Division of Organic Chemistry
CSIR-National Chemical Laboratory
Dr. Homi Bhaba Road, Pune-411 008,
INDIA

Abstract

The enhanced permeability and retention (EPR) effect is a unique phenomenon of solid tumors related to their anatomical and pathophysiological differences from normal tissues. In solid tumors, angiogenesis leads to high vascular density. Large gaps exist between endothelial cells in tumor blood vessels, which lead to selective extravasations and retention of macromolecular drugs. This EPR effect served as a basis for development of macromolecular anticancer therapy. There are various factors, which lead to a significantly increased EPR effect and enhanced antitumor drug effects as well. This review discusses the unique anatomy of tumor vessels, molecular mechanisms of factors related to the EPR effect and the role of the EPR effect in the intra-tumoral delivery of protein and peptide drugs, macromolecular drugs and drug-loaded long-circulating nanocarriers.

Keyword: Enhanced permeability retention effect; Passive targeting; Tumor targeting; Nanocarriers

Introduction

The enhanced permeability and retention (EPR) effect was first reported by Matsumura and Maeda in 1986 [1]. It is a phenomenon wherein certain sizes of molecules (macromolecular bioactive compounds or nanoparticles) accumulate in tumor tissue much more than they do in normal tissues. This phenomenon can be generally explained as that, so as to grow very fast, tumor cells must stimulate the production of new blood vessels. EPR effect is an exceptional property of solid tumors associated to their pathophysiological and anatomical variations from normal tissues. The accumulation and retention of macromolecules are very much improved in tumor tissue as compared to those in normal tissue. This phenomenon is mainly pertinent only to macromolecules and particles and not to low-molecular-weight compounds [2].

It is very well-established phenomenon that under certain pathological condition (inflammation/hypoxia and tumors) the endothelial lining cells of the blood vessel turn into more permeable than the normal state. Consequently, particles ranging from ~50 to 500 nm in size, leave the vascular bed and gather inside the interstitial space. This phenomenon has been demonstrated in a variety of tumors [3]. If these particles are loaded with a bioactive molecules or anti-cancer drugs, the bioactive molecule can be eventually delivered from the particles to tumor [4]. The leaky tumor vasculature permits macromolecules and nanocarriers to come into the tumor interstitial space, whereas due to the compromised lymphatic filtration they stay there [5]. On the other hand, “small” low-molecular weight bioactive agents are not retained and return to the circulation by diffusion [6]. EPR-mediated drug delivery have been considered as an effective way to deliver chemotherapeutic drugs into the tumors, mainly macromolecular drugs and drug-loaded nanocarriers [7]. EPR effect may result in macromolecules accumulation at much higher concentrations in tumor tissues as compared to normal tissues. The majority of stealth nanocarriers accumulate in tumor tissue at a concentration 5–10 times higher than that of plasma after 24 h of intravenous injection, in addition more than 10 times higher than that of normal tissue [8].

Macromolecular delivery systems have been explored for the delivery of antineoplastic agents using EPR effect. The concept behind utilization of drug-macromolecule conjugates is to improve distribution of the drug [9, 10]. Similarly, nanocarriers, such polymeric nanoparticles have been explored tremendously for drug delivery to tumors via passive accumulation using EPR effect. This review shade light on the distinctive composition of tumor vessels, molecular factors related to the EPR effect, and the utility of the EPR effect in the intra-tumoral delivery of nanocarriers,.

Distinctive Characteristics of Tumor Blood Vessels

The EPR effect is a exceptional phenomenon of solid tumors associated to their anatomical and pathophysiological variations than normal tissues.

Morphology of tumor blood vessels

Unlike normal tissues and organs, the solid tumors show hypervasculature, particularly when tumors are small, some exceptions being pancreatic, prostate and large metastatic liver cancers [11]. Tumor angiogenesis is most important aspect that causes rapid tumor growth, which begin as the tumor diameter becomes approximately 0.8–1.0 mm [12]. As shown in Figure. 1, the newly formed tumor blood vessels generally have an anomalous structural design, which include defective endothelial

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cells with large fenestrations, lack of a smooth muscle layer, wide lumen, asymmetrical vascular configuration as well as impaired receptors for angiotensin II (AT-II) [13, 14]. Additionally, in these vessels blood flow direction is also irregular or inconsistent [15].

Blood vasculature and tumor blood flow

The arteriole–venule (A–V) pressure difference and flow resistance determines the blood flow within a tissue. There are very few arteries/arterioles in tumor periphery and blood vessels in the tumor interior are mainly veins/venuoles. Thus, the A–V pressure difference is insignificant in the central region but greater in the tumor periphery. This to a degree clarifies the higher blood flow in the periphery and the lower blood flow in the center of a tumor. Blood vessel distribution within a tumor depends on the tumor size and the locations within a tumor. Small tumors are perfused by vasculature derived from adjacent host tissues, while bigger tumors are typically accompanied by newly formed microvessels [16]. A solid tumor encompasses three regions: (a) avascular necrotic area with no vasculature, (b) stably perfused region containing many venous vessels and few arteriolar vessels, and (c) semi-necrotic region containing capillaries. Vascularization is inversely correlated to tumor size wherein bigger tumors exhibit a higher ratio of avascular to perfused areas with greater distances between capillaries [17].

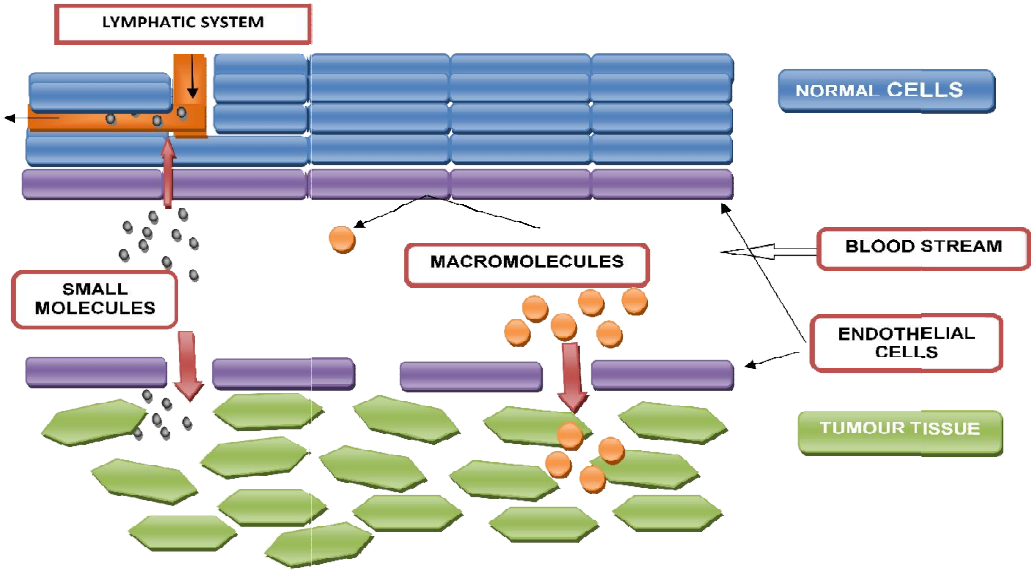

Figure. 1: Anomalous structural of newly formed tumor blood vessels.

The intra-tumoral vasculature heterogeneities play a major role in uneven anti-cancer drug distribution within solid tumors. It has been reported that in transplanted rodent tumors, the pore size of tumor microvessels differs from 100 to 700 nm in diameter based on the anatomical location. The enhanced vascular permeability and dilatation in tumors is attributed to elevated levels of vasoactive and growth factors (vascular endothelial growth factor, nitric oxide, bradykinin, basic fibroblast growth factor) [16]. Due to leaky vessel, the key trail of macromolecules transport across tumor microvascular wall is by extravasation via diffusion and/or convection through the discontinuous endothelial junctions. Extravasation of molecules is related to fluid exchange across vasculature wall, which is based on the hydrostatic pressure gradient amid intravascular space (i.e., microvascular pressure or MVP) and interstitial space (i.e., interstitial fluid pressure or IFP) and the osmotic pressure gradient due to variations in protein levels [18].

Impaired lymphatic clearance

Lymphatic vessels are more permeable to fluid and solutes as compared to blood capillaries and widely distributed throughout the body. The key role of the lymphatic system is to return the interstitial fluid to the blood circulation [19]. Lymphatic clearance of tumor tissue and lymphatic metastasis tumor tissues typically lack efficient lymphatic drainage, which decreases clearance of nanocarriers from tumor interstitium [20]. Lymphatic system is the most important route for metastasis of tumor cells into normal tissues. Lymphatic metastasis is one of the most frightening consequences of cancer progression, and its control is critically important [21]. Maeda *et al.* have shown that evans blue-albumin complex when injected i.v. into tumor-bearing mice it accumulated and stayed in the tumor for above a week, conversely it was gradually cleared from non-tumor tissue by normal lymphatic function [22].

The Factors Which Affect EPR Effect

Tumors overexpress many permeability-enhancing factors, which contribute to an enhanced EPR effect. It is also affected by many pathophysiological factors involved in enhancement of the extravasation of macromolecules. Few factors are given below.
Active angiogenesis and high vascular density
Extensive production of vascular mediators that facilitate extravasation, including
Prostaglandins
VEGF
Nitric oxide
Bradykinin
Peroxynitrite
Collagenase

Augmented Tumor Delivery of Polymer Conjugates and Nanocarriers via EPR Effect

Polymer-drug conjugates

Polymer conjugates are nano-sized hybrid constructs that covalently join a bioactive molecule with a polymer. This ensure efficient delivery and availability of bioactive molecule to the required intracellular compartment [23]. It has been demonstrated previously that polymer conjugation uphold tumor targeting by the EPR effect and, allows lysosomotrophic drug delivery. Polymer conjugates have the potential to improve the treatment of drug-resistant tumors with reduction in toxicity [24]. These type of polymer-drug multicomponent conjugates have been already transferred to clinics [25]. Polymer-protein as well as polymer-drug conjugates were employed as a one of the foremost classes of anticancer nanomedicines [24]. Polymer conjugates offer a lot of advantages with regards to EPR effect such as Stealth character (if conjugated with PEG), reduced immunogenicity, prolonged circulation and thus half life, enhanced stability and higher cellular uptake [26, 27]. In addition EPR effect, intracellular uptake of polymer-drug conjugated can be enhanced by exploiting receptor-mediated endocytosis wherein targeting ligands can be attached to another end of the polymer-drug conjugate [9, 28].

Nanocarriers

EPR based tumor targeting necessitates drug delivery systems to be long-circulating so that a sufficient level can accumulate in the target. Modifying surface of the nanocarriers with water-soluble polymers (e.g. PEG) is most common way to keep them in the blood for long time [29, 30]. The size and long circulation of a nanocarrier play very crucial role in EPR-mediated drug delivery [31, 32]. Similarly, properties of delivery system like molecular weight, surface characteristics and surface charge also play very important role for good EPR effect.
Delivery systems that have molecular weight of more than 30 KDa can escape quick renal clearance and keep circulating in blood but smaller molecules that readily redistribute to blood circulation via diffusion and/or convection [33]. Likewise, The size of the total complex should not be more than 200 nm. This is because the delivery system must be able to penetrate the openings or fenestrae of the endothelial cells of the capillaries. The nature of the surface of the delivery system should be hydrophilic to avoid removal by the monophasic phagocytic system (MPS) [34]. In addition, surface charge of the drug delivery system has a positive or negative charge or is neutral in charge indicates for how long it can circulate in the blood [35, 36], because the luminal endothelial membrane in vessels is negatively charged and therefore can be targeted by cationic NP through electrostatic interactions [37-39]. This approach leads to more rapid and more extensive NP extravasation and retention in tumors relative to passive targeting via the EPR effect [40].

Polymeric nanoparticles

Stealth long-circulating nanocarriers, for example polymeric nanoparticles, are competent of accumulating in different pathological regions using the EPR effect owing to altered vasculature, and have been exploited much for anti-cancer drug delivery to tumors by means of passive accumulation. Polymeric nanoparticles exhibit dose-independent, log-linear and non-saturable kinetics with increased bioavailability [41, 42]. Hydrophobically modified glycol chitosan (HGCs) nanoparticles loaded with cisplatin, effectively accumulated in tumor tissues in tumor-xenografted mice, on account of prolonged circulation and EPR effect [43]. PEG conjugation on nanoparticles reported to prolonged half life in the blood compartment which allowed nanoparticles to selectively extravasate in pathological areas such as tumors or inflamed regions [44]. Mitraa *et al* [45] reported improved therapeutic efficacy of doxorubicin coupled dextran (DOX-DEX) when encapsulated in biocompatible, biodegradable and long circulating hydrogel nanoparticles of 100±10 nm size, which favored the EPR effect. Etame *et al* [46] reported gold nanoparticles (AuNPs) with potential relevance for brain tumor targeting wherein they designed and characterized AuNPs having diverse particle sizes of the core (4–24 nm) and PEG chain lengths (Mw 1000–10,000 Da). The authors established that the permeation properties of the nanoparticles are size-dependent relating to the core particle size and PEG chain length. Their result suggested that smaller PEG chain formulations (e.g. PEG 1000 and 2000) were more easily transported as compared to higher PEG chain formulations (e.g. PEG 5000 and 10,000).
Nanoparticles are proficient to deliver therapeutic as well as imaging contrast agents safely to tumors for theranoustic application using

EPR effect, wherein both can be encapsulated in the core of nanoparticles for dual purpose [47]. Magnetic nanoparticles (MNPs) also own exceptional magnetic properties with the ability to work at the cellular and molecular level making them an attractive platform as contrast agents for MRI and as drug delivery carriers [48]. Hai *et al* [49] anticipated the co-delivery approach to reduce the quantity of each drug and to accomplish the synergistic effect for cancer therapies. The idea was to overcome the drawback of development of drug resistance and high toxicity. NPs from an amphiphilic copolymer PLGA-mPEG was used to co-deliver hydrophilic doxorubicin and hydrophobic paclitaxel. Cellular uptake studies demonstrated that both drugs were efficiently taken up by the cells and co-delivery indicating a synergistic effect.

Liposome

Liposomes, or phospholipid vesicles, have been well renowned as a prospective drug delivery carrier for more than three decades. These nanocarriers display better extravasation and accumulation in solid tumors owing to enhanced endothelial permeability and reduced lymphatic drainage (EPR effect) [50]. These provide a controlled release carrier as well as a biocompatible solubilizing nanocarrier for inadequately soluble drugs. On account of their size, which varies from 50 to 250 nm can be used for the systemically administered wherein they exhibit some distinctive pharmacokinetic characteristics [51, 52]. These include non-recognition from the RES resulting in long circulation time with less hepatic as well as splenic distribution [53]. Liposomes have shown exceptional qualities for improvement of drug loading, as stealth liposomes [54], pH-sensitive liposomes for cytosolic drug delivery, cationic liposomes for nucleic acid delivery, targeted liposomes for selective delivery to specific tumor [55] and temperature-responsive liposomes for hyperthermia [56].

Plasma proteins are reported to adsorbed easily on the surface of conventional liposome resulting in recognition by the RES, and eliminated from the body [57]. PEGylated liposomes with extended circulation time in the blood are commonly called “stealth” or sterically stabilized liposomes [58, 59]. PEG-liposomes escape from the wide gaps between neighboring endothelial cells and widely penetrate into the interstitial space of tumor. Their specificity has been distinctly enhanced by using ligands. Among the different techniques for active targeting, immunoliposomes by means of an antibody as targeting ligand have attracted a lot of attention. PEG coating can also be detached using local pathological conditions (e.g. lower pH of tumors) [60, 61].

Mayer *et al.* [62] have examined the application of liposomes as a co-delivery vehicle for combinations of cisplatin/daunorubicin, daunorubicin/cytarabine, and irinotecan/fluoxuridine. Long circulating stealth formulation of DOX has exhibited increased solid tumor accumulation because of the EPR effect with decreased cardiac toxicity relative to the free DOX [54]. In an another study, co-encapsulation of DOX and verapamil in liposomes was shown to be highly effective against multidrug resistant cancer cells [63]. DOX loaded PEG-coated liposomes have demonstrated very high efficiency in EPR-based tumor therapy and strongly reduces the side effects [64, 65]. Clinical data also suggested the striking effect of DOX-PEG-liposomes against hepatocellular carcinoma [66]. PEGylated liposomes loaded with rapamycin avoided the major sequestration of rapamycin into the erythrocytes [67]. Photodynamic therapy (PDT) is a rapidly-developing technique for the management of superficial tumors. In PDT, liposomes have been used as drug carriers as well as enhancers both [68]. Vascular permeability inside the tumor significantly influence the accumulation of PEG liposome in tumor and is one of the vital components for *in vivo* anti-tumor effectiveness of DOX-PEG-liposomal [69]. Man *et al* [70] reported treatment of trastuzumab-insensitive breast cancer by development of a liposome formulation containing a combination of vincristine and quercetin for synergistic effect.

Dendrimer

Dendrimers are well-organized, highly branched nanostructures which provide a series of flexible chemical alteration for a variety of purposes [71-73]. The biological fate of dendrimer loaded drugs can be considerably changed owing to their physicochemical properties. Pharmacokinetics play an key function for successful *in vivo* application and clinical translation of dendrimer [74]. Dendrimer nanoconstructs explore innovative promising class of nanocarriers as delivery and imaging agent via both passive and active targeting approaches. PEGylation can also increase circulation time of dendrimer [75], Dendrimer have resulted in prolonged plasma exposure of DOX as compared to f free drug. In addition, tissue biodistribution profiles of PEGylated dendrimer revealed more effective tumor targeting of drug. The noteworthy antitumor activity of dendrimer–DOX was due to the ability of the dendrimer to favorably modulate the pharmacokinetics of loaded DOX [76]. The PEGylated dendrimers with the higher generation and the longer PEG led the greater blood retention [77]. Okuda *et al* [78] evaluated biodistribution characteristics of PEGylation dendrimers in both normal and tumor-bearing mice and demonstrated effective accumulation in tumor tissue via the EPR effect. Dendrimers and dendrimer-based therapeutics are outstanding candidates in therapy and diagnostics field as more and more biological systems have benefited from these starburst molecules. Dendrimer conjugates have huge potential to serve as an innovative multifunctional delivery and imaging agent for the treatment and detection of metastatic tumors [79].

Carbon nanotubes

Carbon nanotubes (CNTs), have turn out to be a popular nanocarrier due to their unique physicochemical properties for cancer diagnosis and therapy. They are considered to be very promising nanomaterials for delivering drugs or small therapeutic molecules [80]. In the last few years, CNTs have been exploited for cancer treatment modality, including lymphatic targeted chemotherapy, photodynamic therapy, drug delivery, thermal therapy as well as gene therapy [81]. Zhuang *et al* (2008) reported that, PEGylated single-walled carbon nanotubes (SWNT) showed higher efficacy in suppressing tumor growth, due to prolonged blood circulation and EPR effect [82]. Cheng *et al* [83] reported PEGylated MWCNTs as drug carrier to overcome multidrug resistance. Their results also concluded that PEGylated MWCNTs are efficient drug carriers to conjugate drugs for overcoming MDR in cancer treatment. Owing to their unique surface properties, large surface areas and needle-like shape, can transport a higher amount of bioactive agents, including DNA and RNA, to the target disease sites [84]. Functionalized carbon nanotubes also exhibit distinctive properties that enable a range of biomedical applications, including the diagnosis and treatment of cancer [85]. Carbon nanotubes can be also used as a pro-drug by attaching pro-drugs to different parts of carbon nanotubes, wherein once the drug-loaded nanotube is into a cancer cell via EPR effect, the pro-drug is metabolized into its toxic form, and thus kill the cancer cell [86].

Conclusion

The EPR effect is the exclusive and most critical event that is occurring in tumor tissues, which accounts via pathophysiological and anatomical features of tumor blood vessels. Moreover, vascular mediators, such as NO, BK and PGs critically affect EPR effect. Owing to EPR effect, macromolecular anticancer drugs are getting additional attraction in cancer chemotherapy as compared to conventional chemotherapy which can certainly improve therapeutic efficacy and reduce adverse effects. EPR effects is observed solitary for polymer-drug conjugates as well as nanocarriers of specific size. Nowadays it turn out to be possible to accumulate nanocarriers selectively in tumor either by altering vascular mediators or by increasing the systemic blood pressure via infusion of angiotensin II. This enhanced vascular permeability of tumor tissue is the most important factor to be considered for development for highly selective targeting to the specific desired tumors.

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Conflict of Interest

The authors declare no conflict of interest.

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